Skin Sensitization Potency Assessments of Fragrance Materials using the GARDskin Dose-Response Assay

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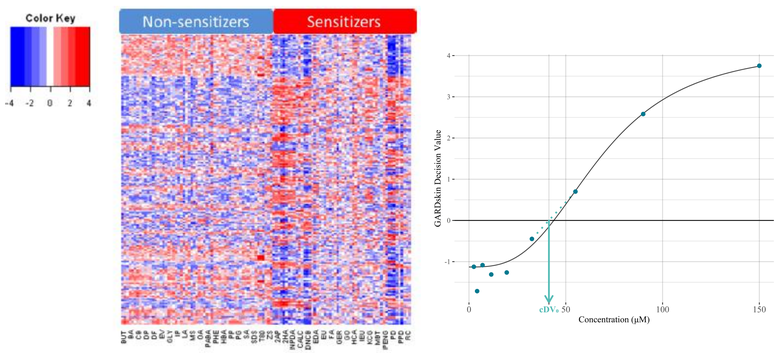
Abstract/Introduction

Several New Approach Methods for hazard identification of skin sensitizers have been developed and incorporated as OECD Test Guidelines. However, the methods for potency assessment are still lacking. GARDskin (OECD TGP 4.106) was initially developed to identify skin sensitizers by monitoring transcriptional patterns of a biomarker signature in a dendritic-like cell line. The predictive capacity of GARDskin has been demonstrated previously, with 95.8% accuracy, 91.7% positive predictive value, and 100.0% negative predictive value (1 false positive, n = 24) (Johansson, Gradin et al. 2019). To derive potency information, a strategy based on dose-response measurements in GARDskin, referred to as the GARDskin Dose-Response assay, has recently been proposed. The readout of the assay corresponds to the lowest concentration required to exceed the binary classification threshold in GARDskin. This concentration correlates with local lymph node assay (LLNA) EC3 and human no observed effect level (NOEL) values and linear regression models have been established to exploit these relationships for potency predictions. In this blinded study, 12 fragrance materials (10 very weak sensitizers and 2 weak sensitizers) were evaluated in GARDskin Dose-Response. Results were evaluated by comparing predicted values to the reference potency categories. Three of the very weak sensitizers were predicted as non-sensitizers by GARDskin Dose-Response. For the remaining nine materials which were predicted as sensitizers, the predicted EC3 and NOEL values closely approximated the reference data for most materials. Based on results from this dataset, **GARDskin Dose-Response appears useful for potency assessment for weak sensitizers** and may constitute a promising strategy for deriving a point-of-departure for quantitative risk assessments.

Materials and Methods

GARDskin Dose-Response

In this blinded study, each of the fragrance materials was applied to the SenzaCells at ≥6 different concentrations for 24 hours. Gene expression levels of 200 biomarkers for skin sensitization were measured (Johansson, Albrekt et al. 2013). At each concentration, the induction of skin sensitization is determined using Support Vector Machine for supervised classification. To that end, a decision value (DV) was generated for each concentration and the lowest concentration required to induce a positive response (cDV0) was identified. This concentration was subsequently used to predict an LLNA EC3 value or human a NOEL value using the a priori established linear regression models.



Materials and Methods (cont.)

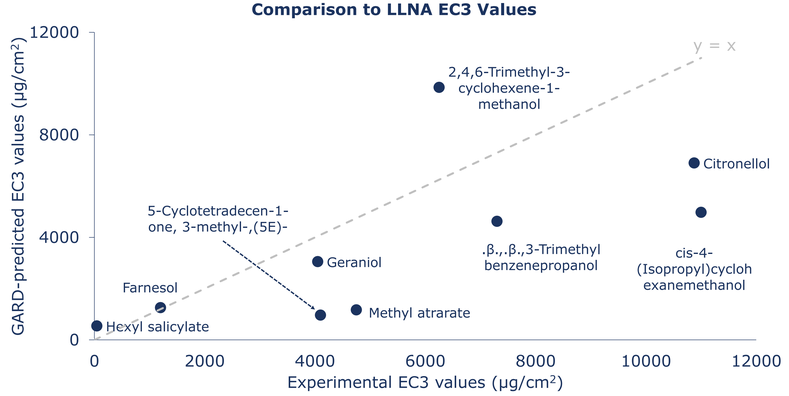
Benchmark Data

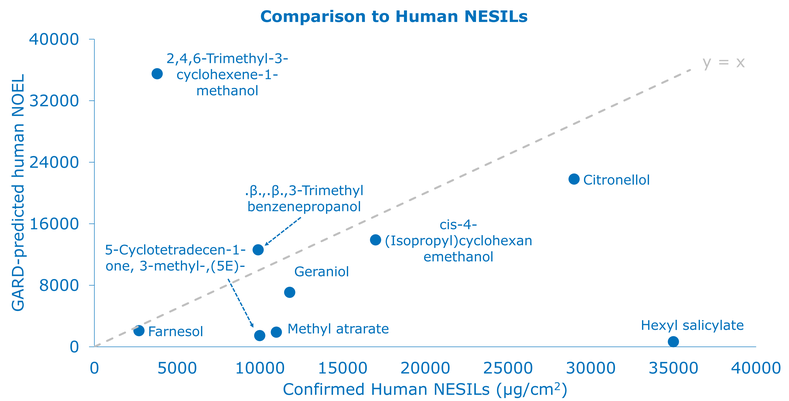
Potency prediction by GARDskin Dose-Response was compared to the LLNA EC3 value and human No Expected Sensitization Induction Level (NESIL) values, obtained from RIFM Database (www.RIFM.org).The potency of 12 fragrance materials were determined using a weight of evidence (WoE) approach, by collectively considering all available human, animal, *in vitro*, *in chemico*, and *in silico* data on these materials. This WoE approach, as well as the data that were used for potency categorization are described in Na et al., 2021 (manuscript under review). For the potency evaluation, the concentrations were converted to dose per unit area of skin (µg/cm²).

Potency Category	Dose Range* (µg/cm²)	LLNA EC3 Dose Range† (µg/cm²)
Extreme	<25	<25
Strong	25-500	25-<250
Moderate	500-2500	250-<2500
Weak	>2500-10000	2500-25000
Very Weak	>10000	
Non sensitizer	Negative	

*Adapted from Api et al. (2017) †Adapted from ECETOC (2003)

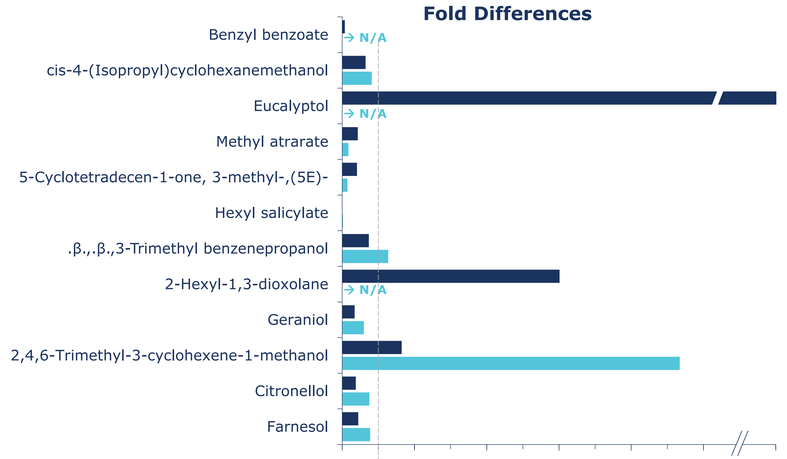
Results





Results/Conclusion

For 10/12 of the tested fragrance materials, the prediction by GARDskin Dose-Response was comparable to that of the LLNA.



GARDskin Dose-Response closely approximated the potency categories of 9/12 fragrance materials tested.

Name	CAS No.	WoE Category	Predicted Human Potency Category (GARD)
Farnesol	4602-84-0	Weak	Moderate
Citronellol	106-22-9	Very Weak	Very Weak
2,4,6-Trimethyl-3-cyclohexene-1-methanol	68527-77-5	Weak	Very Weak
Geraniol	106-24-1	Very Weak	Weak
2-Hexyl-1,3-dioxolane	1708-34-5	Very Weak	NS
.β.,.β.,3-Trimethyl benzenepropanol	103694-68-4	Very Weak	Very Weak
Hexyl salicylate	6259-76-3	Very Weak	Moderate
5-Cyclotetradecen-1-one, 3-methyl-,(5E)-	259854-70-1	Very Weak	Moderate
Methyl atrarate	4707-47-5	Very Weak	Moderate
Eucalyptol	470-82-6	Very Weak	NS
cis-4-(Isopropyl)cyclohexanemethanol	13828-37-0	Very Weak	Very Weak
Benzyl benzoate	120-51-4	Very Weak	NS

Exact match | Approximate | Off

References

Api, A. M., R. Parakhia, D. O'Brien and D. A. Basketter (2017). "Fragrances Categorized According to Relative Human Skin Sensitization Potency." Dermatitis **28**(5): 299-307.
ECETOC (2003). Contact Sensitization: Classification According to Potency Technical Report No. 87.
Johansson, H., A.-S. Albrekt, C. A. K. Borrebaeck and M. Lindstedt (2013). "The GARD assay for assessment of chemical skin sensitizers." Toxicology in Vitro **27**(3): 1163-1169.
Johansson, H., R. Gradin, A. Johansson, E. Adriaens, A. Edwards, V. Zuckerstätter, A. Jerre, F. Burleson, H. Gehrke and E. L. Roggen (2019). "Validation of the GARD™skin Assay for Assessment of Chemical Skin Sensitizers: Ring Trial Results of Predictive Performance and Reproducibility." Toxicological sciences : an official journal of the Society of Toxicology **170**(2): 374-381.

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